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THE CHROMOSOME COMPLEX OF MELANOPLUS BIVITTATUS SAY.

BY NADINE NOWLIN.

(Contribution from the Zoölogical Laboratory, No. 179.)

Plates XVI, XVII.

THAT certain chromosomes are distinct individuals and maintain their identity through the different generations from spermatogonia to spermatids there can now be no doubt. The work on the accessory chromosome, and very recently that on the idiochromosomes, has furnished specific evidence of this. Moreover, the complex as a whole has been studied and the individuality of most of the chromosomes of the group has been fairly well established. For instance, Sutton ('02) carefully measured the chromosomes of the *Brachystola magna* complex. Baumgartner ('04), in his work on the cricket germ-cell, has laid stress on the individuality as expressed in form, and Boveri ('02) showed experimentally a difference in function. Perhaps the most conclusive evidence of chromosome individuality is the occurrence of the same number in an entire family, as found by McClung ('05) in the *Acrididæ*.

Chromosomes may exhibit individuality then (1) in size, (2) in form, (3) in function, (4) in constancy in number. To these might now be added another, (5) individuality expressed in grouping. McClung ('05), in his recent work on certain species of *Hesperotettix* and *Mermiria*, discovered a grouping or association of definite chromosomes that holds not only for the individuals of a species, but for all the species of the genus thus far investigated. Such an aggregation of chromosomes he calls a *multiple*, and by careful measurement of the elements of the multiple he finds that the specific difference

is one of size. The importance of the discovery of the multiple chromosome is very evident: the elements composing it can be followed through the different species without the shadow of a doubt, which is most important in determining its bearing on heredity.*

The crying need just now is for a better understanding of the chromosome complex, not only of one species but of many species of a genus, so that careful comparisons can be made. This is the first step toward a correlation of chromosomes and body characters. If chromosomes are definite masses endowed with the transmission of characteristics, then the complex should not differ greatly in the various species of a genus.

This paper is the first of a series which will have for its object the investigation of the chromosome complex of the genus *Melanoplus,* and forms part of a plan of investigation into the acrididean complex which has been outlined by Dr. C. E. McClung. The genus *Melanoplus* is a large and variable one, while *Hesperotettix* is small and well defined. Since these belong to the same group, the *Melanopli,* the nature of the complex may be correlated with the generic variations.

A species of *Melanoplus* has been investigated by Wilcox ('95), but his aims were somewhat different from those of the present paper, as were his results. The old idea was that an understanding of the complex of one species meant not only knowledge of the whole genus but of practically all animals. Thus, believing that the first division in *Melanoplus femur-rubrum* is transverse, he concludes that this method of division is general. In such a well-defined group as *Hesperotettix* (McClung, '05) it was found that what is true for one species may be true for all; but in *Melanoplus,* a large and variable group, there are probably variations to be found in size, in chromosome grouping, and in various other respects. The object of this series is to study and compare these variations. As to division, however, no discrepancies have been discovered. McClung found, contrary to Wilcox, that the first division in *Melanoplus femur-rubrum* is longitudinal, and my investigations on *M. bivittatus* supports this view.

* Since this paper was written, Doctor Wilson ('07) has found multiples in the Hemiptera.

MATERIAL AND METHODS.

The form under consideration, *Melanoplus bivittatus* Say, belonging to that group of hoppers called *Acridiinæ*, was collected in Graham county, western Kansas, in the summer of 1904, and forms part of an extensive collection brought together for the purpose of such studies as the present. For both material and direction in this work I am indebted to Dr. C. E. McClung.

The testes were fixed in Flemming's fluid and stained in Heidenhain's iron-hæmatoxylin. Sections were cut six micra in thickness.

OBSERVATIONS.

The germ-cells of *Melanoplus*, as of all other genera of the *Acrididæ* thus far investigated, have twenty-three spermatogonial chromosomes, twenty-two of which may be paired and the twenty-third without a mate, the typical accessory of McClung. Due to the great crowding in the spermatogonia the exact form of the individual chromosomes is difficult to see, but the general shape is that of a rod. The smallest pair, however, is without exception spherical. The chromosomes are arranged radially around an open center in which occasionally lie from one to two chromosomes (fig. 1), and can with a fair degree of accuracy be paired. The complex is composed of a graduated series of chromosomes whose size relations are well shown in plate XVII.

In this material the lateral view of the first spermatocyte metaphase is more satisfactory for a comparative study of the chromosomes than the polar view. Since practically all the chromosomes form like tetrads, they cannot with any accuracy be distinguished by form, so here the size test becomes necessary. A glance at figures 3A and 3B will show the great variation in size of the chromosomes of a complex.

For convenience it has been decided to number the chromosomes according to their size, calling the smallest No. 1 (fig. 3). This small chromosome assumes the form of a cross at one stage in its division but completes its changes before reaching the metaphase. It has never been observed in any but spherical form here. There is another chromosome which seems to do the same: No. 11, in the metaphase, is always pulled out into the long rod-like form. Running down column 11, plate XVII, one sees this without exception. No. 12 is the largest of

the group, and in its fully extended state measures about eight times the length of the smallest. This chromosome stands out prominently through all the generations from spermatogonia to spermatids and will serve excellently for comparison in other species. Chromosome No. 10 is characterized by the form of a ring and is easily distinguished in the first division, both in a lateral and a polar view. The single ring seems to be constant for this species. Occasionally the largest two chromosomes assume the ring shape (fig. 9), and on the other hand cells are found which contain no rings (fig. 6). The formation of the ring is exactly like the other chromosomes of this group, a bivalent chromosome possessing, when it comes into the spindle, a longitudinal split along which it divides in the first spermatocyte. The chromosomes numbered 9, 8, 7, show a gradual diminution in size but have no peculiarity of form to distinguish them. Chromosome No. 6 does not differ in form, but, as is characteristic of the accessory, can be recognized by its eccentric position in the spindle. The next four chromosomes are not distinguishable by shape, but No. 1 is always spherical. No. 2 is usually similar to No. 1 in form but slightly larger, and is sometimes found in the form of a cross (pl. XVII, cell F, row 2).

There are, then, five chromosomes of this group that can be recognized with certainty in the spermatocyte generations: No. 1, the small spherical; No. 6, the accessory; No. 10, the ring; No. 11, the long chromosome, always fully extended and next largest, and No. 12, the largest chromosome of the complex.

Despite the crowding in the spermatogonia, two pairs of chromosomes, No. 12 and No. 1 (fig. 1), can be easily distinguished; the others are doubtful.

The chromosomes divide longitudinally in the first division, with the exception of the accessory, which passes undivided to one pole. As some dispute has arisen concerning this division it might not be amiss to follow out briefly the behavior of the chromosomes from the spermatogonial stage. Here we find the chromosomes often showing the longitudinal split (fig. 1, chr. 9). In the early growth stages the spireme is distinctly split, and even after segmenting and much condensing this division is still visible. Very soon after synapsis the bivalent chromosome begins to separate into halves along the longi-

tudinal split. One program of movement is closely adhered to in every case. The longitudinal split runs from one end of the bivalent chromosome to the other, and separation along this split begins at the place of union of the two univalent elements, as shown in figure 2, chromosome 4. It is in this condition that the chromosomes sometimes enter the spindle, their long axis, or the longitudinal split, parallel with the equator (pl. XVII, cell G, row 7). More often, however, division has continued to the point where the arms of the cross are equal (pl. 2, cell A, row 8), or even farther, as in cell A, row 9. The chromosomes of this complex divide very irregularly as to time, some having almost completed the separation while others are but beginning. This variation is seen in plate XVII, cell G. Chromosome 11 seems to be precocious. The ring chromosome, No. 10, divides slowly, as might be expected, and, like the others, shows the first division to be longitudinal. Its behavior is entirely different from such rings as are found in the beetles, where the two bivalent elements separate in the first spermatocyte (Stevens, '06; Nowlin, '06).

The ring has some significance in this material—not that its occurrence is unique, but because of the constancy of this form of chromosome for different species. A single ring seems to be constant for *Melanoplus bivittatus,* while in one of the Tryxalines, *Syrbula admirablis,* now under investigation by Mr. Robertson, there are five rings.

There is little difficulty in seeing that a chromosome which enters the spindle with its longitudinal split at right angles to the long axis of the spindle divides longitudinally in the first spermatocyte. The spindle fibres are attached at right angles to the split (pl. XVII, cell G, row 7) and consequently pull the halves apart along this split. But a chromosome like No. 12, which enters the spindle with its longitudinal split parallel with the long axis, may cause confusion. On account of its great size this chromosome can be easily followed through the different generations. In a polar spermatogonial view (fig. 1, chr. 12) it is seen to be long and rod-shaped, about eight times the volume of the smallest, No. 1. In the early prophase we recognize it again by the great size (fig. 2). In the late prophase we see it in the form of a cross (fig. 8), and in metaphase (fig. 10A, chr. 12) again as a rod. There is but one explanation: the chromosome during the prophase begins to

separate into identical halves along the longitudinal split and practically completes the movement by the time it enters the spindle. No. 12 never seems to vary from this behavior. All that remains to complete the division of No. 12 is separation of the ends, and as soon as this occurs the chromatids spring apart along the former longitudinal split and pass to the poles as V's. This is plainly seen, too, in a polar view of the second spermatocyte (fig. 13A, chr. 12). This division is transverse and consists of a separation of paternal and maternal elements at the point of union during synapsis.

In the first spermatocyte division the accessory passes undivided to one pole and as a consequence we get eleven chromosomes in one half of the second spermatocytes and twelve in the other half (figs. 13, 14). The distinctions between chromosomes made in the first spermatocyte can be carried out in the second. The volume is of course reduced half (figs. 13A, 13B, 14A, 14B). The ring cannot be distinguished by form, but its size ranks it about 10.

A comparison of figures 11, 12 and 13 will show the relative size of the chromosomes of the first and second spermatocytes. Figures 13 and 14 show the constancy in size and shape of the chromosomes of the second spermatocyte.

SUMMARY.

1. The chromosomes of this complex form a graduated series, the longest being nearly eight times the volume of the smallest.

2. All the chromosomes, with the exception of the accessory, form true tetrads and divide longitudinally in the first and transversely in the second spermatocyte. The accessory divides longitudinally in the second spermatocyte.

3. There are in this species no multiples or any other differential chromosomes than the accessory, whose function and history are so well known from the works of McClung and others.

4. At least five pairs of the chromosomes of this complex can be recognized in the different cell generations. This complex will be used as the basis for comparison in the other species of this genus now under investigation.*

* In justice to the author it should be stated that no opportunity was afforded her to read the proof of this article.—C. E. M.

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